MOLECULAR AND CELLULAR BIOLOGY, July 1998, p. 3851–3861 Vol. 18, No. 7
0270-7306/98/$04.00+0

Differential Utilization of Ras Signaling Pathways by Macrophage Colony-Stimulating Factor (CSF) and Granulocyte-Macrophage CSF Receptors during Macrophage Differentiation

FABIEN GUIDEZ,† ANDREW C. LI, ANDREW HORVAI, JOHN S. WELCH, AND CHRISTOPHER K. GLASS*

Divisions of Endocrinology and Metabolism and Cellular and Molecular Medicine, Department of Medicine, University of California, San Diego, La Jolla, California 92093-0651

Received 13 October 1997/Returned for modification 26 November 1997/Accepted 27 March 1998

Granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) independently stimulate the proliferation and differentiation of macrophages from bone marrow progenitor cells. Although the GM-CSF and M-CSF receptors are unrelated, both couple to Ras-dependent signal transduction pathways, suggesting that these pathways might account for common actions of GM-CSF and M-CSF on the expression of macrophage-specific genes. To test this hypothesis, we have investigated the mechanisms by which GM-CSF and M-CSF regulate the expression of the macrophage scavenger receptor A (SR-A) gene. We demonstrate that induction of the SR-A gene by M-CSF is dependent on AP-1 and cooperating Ets domain transcription factors that bind to sites in an M-CSF-dependent enhancer located 4.1 to 4.5 kb upstream of the transcriptional start site. In contrast, regulation by GM-CSF requires a separate enhancer located 4.5 to 4.8 kb upstream of the transcriptional start site that confers both immediate-early and sustained transcriptional responses. Results of a combination of DNA binding experiments and functional assays suggest that immediate transcriptional responses are mediated by DNA binding proteins that are constitutively bound to the GM-CSF enhancer and are activated by Ras. At 12 to 24 h after GM-CSF treatment, the GM-CSF enhancer becomes further occupied by additional DNA binding proteins that may contribute to sustained transcriptional responses. In concert, these studies indicate that GM-CSF and M-CSF differentially utilize Ras-dependent signal transduction pathways to regulate scavenger receptor gene expression, consistent with the distinct functional properties of M-CSF- and GM-CSF-derived macrophages.

Macrophages originate from multipotent progenitor cells in bone marrow and serve critical physiological roles in host immunity, wound healing, and inflammation (10). The colony-stimulating factors (CSFs), through a network of cell surface receptors, cytoplasmic signaling molecules, and transcription factors, regulate macrophage differentiation and function (23). Macrophage CSF (M-CSF) and granulocyte-macrophage CSF (GM-CSF) were originally identified based on their ability to promote macrophage growth and differentiation in colony-forming assays using primary bone marrow progenitor cells (6, 40). M-CSF exerts effects on gene expression through a specific, high-affinity M-CSF receptor encoded by the c-*fms* proto-oncogene (36, 38, 39). The binding of M-CSF to this receptor leads to dimerization and autophosphorylation of tyrosines within the cytoplasmic tail, resulting in the activation of multiple intracellular signaling pathways, including Ras, phosphatidylinositol 3-kinase, and phospholipase C, via the docking of specific SH2 domain-containing proteins (11, 21, 31, 34, 44). Activation of the Ras pathway has been proposed to play an important role in regulating macrophage differentiation by controlling the activities of downstream nuclear targets, including members of the AP-1 and Ets families of transcription factors (12, 17, 18, 49).

GM-CSF, in addition to promoting macrophage differentiation, also promotes the formation of granulocyte and mixed granulocyte-macrophage colonies in soft agar (22). The GM-CSF receptor is related to the cytokine family of receptors, consisting of a specific α subunit and a common β subunit that is shared with the interleukin 3 (IL-3) and IL-5 receptors (15, 24). These receptors lack intrinsic tyrosine kinase activity but interact with and activate Janus kinase 2 (JAK2) in response to the binding of GM-CSF (4, 32). As a consequence, the receptor becomes tyrosine phosphorylated at several locations within the cytoplasmic domain of the β chain and interacts with several specific SH2-containing proteins. The distal C terminus of the β subunit has been demonstrated to couple to Ras-dependent signal transduction pathways (16, 37), leading to activation of AP-1 (1, 50). Membrane-proximal regions of the GM-CSF receptor are involved in the docking and activation of members of the STAT family of transcription factors, including STAT1, STAT3, and STAT5 isoforms (3, 27, 33, 35). While the membrane-proximal region of the β common subunit has been found to be sufficient to mediate proliferative responses to GM-CSF (37, 45), the mechanisms by which the GM-CSF receptor promotes macrophage differentiation remain poorly understood. The observation that both the M-CSF and GM-CSF receptors activate Ras-dependent signal transduction pathways suggests that these pathways may account for at least some of the common effects of M-CSF and GM-CSF on macrophage differentiation.

To examine this question, we have used the scavenger receptor A (SR-A) gene as a model. The SR-A gene encodes a trimeric integral membrane protein that has been proposed to play roles in Ca^{2+}-independent cell adhesion events and in the

* Corresponding author. Mailing address: Division of Cellular and Molecular Medicine, Department of Medicine, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0651. Phone: (619) 534-6011. Fax: (619) 534-8549. E-mail: cglass@ucsd.edu.

† Present address: The Institute of Cancer Research, Royal Cancer Hospital, Chester Beatty Laboratories, London, SW3 6JB, United Kingdom.

uptake of a variety of polyanionic macromolecules, including oxidized low-density lipoprotein (19, 20). Disruption of the SR-A gene in mice results in impaired host responses to some bacterial and viral pathogens and in partial resistance to the development of atherosclerosis (41). The SR-A gene is selectively expressed in macrophages and related cell types and is positively regulated by both M-CSF and GM-CSF (7, 9, 28, 42). Previous studies in monocyte/macrophage cell lines and transgenic mice have demonstrated that expression of the SR-A gene is dependent on the B-cell- and macrophage-specific transcription factor PU.1 (13, 25). In addition, binding sites for members of the Ets family of transcription factors that form ternary complexes with AP-1 proteins are required for activation of the SR-A promoter during macrophage differentiation of THP-1 monocytic leukemia cells in response to phorbol esters (50). Promoter and upstream enhancer elements containing PU.1 and AP-1/Ets binding sites are sufficient to support macrophage-specific gene expression and transcriptional responses to M-CSF in transgenic mice (13, 14).

In the present study, we have investigated the sequences required for transcriptional activation of the SR gene by M-CSF and GM-CSF in transgenic mice and GM-CSF-responsive cell lines. We find that while GM-CSF is capable of transiently activating AP-1 and Ets transcription factors in cell lines, this activity is not sufficient to activate the SR-A gene in primary bone marrow progenitor cells cultured in the presence of GM-CSF. GM-CSF-dependent activation of SR regulatory elements in transgenic mice requires an additional 300-bp enhancer element that is located adjacent to the M-CSF-dependent enhancer 4.5 kb upstream of the transcriptional start site. Twelve hours following GM-CSF treatment, this enhancer region becomes maximally occupied by GM-CSF-induced proteins that are immunologically distinct from STAT1 and STAT5. Intriguingly, the transcriptional activity of the GM-CSF-dependent enhancer is strongly inhibited by dominant negative forms of STAT5 and JAK2, suggesting that at least some of the late effects of GM-CSF on SR-A gene expression are mediated by a set of intermediate transcription factors that bind directly to the GM-CSF-responsive enhancer. Thus, while M-CSF and GM-CSF are both capable of activating AP-1 and cooperating Ets domain transcription factors, they ultimately regulate the expression of the SR-A gene, and presumably other macrophage-specific genes, by different mechanisms. These observations are consistent with the distinct functional properties of M-CSF- and GM-CSF-derived macrophages and with the phenotypic characteristics of macrophage populations observed in M-CSF-deficient mice.

MATERIALS AND METHODS

Construction of SR reporter genes and generation of transgenic mice. The SR-A human growth hormone (hGH) transgenes used in this study are illustrated in Fig. 1A. The silencer-enhancer-promoter (SEP)-hGH and EP (enhancer-promoter)-hGH constructs have been described previously (13, 14). The EXP-hGH transgene was generated by removing the minimal promoter (−245 to +45 bp) from EP-hGH and replacing it with the −696 to +46 promoter. The GMEXP-hGH transgene was generated by replacing the −4.4 to −4.1 kb enhancer in EXP-hGH with the region from −4.8 to −4.1 kb. DNA microinjection, screening, breeding of founder animals, and measurement of hGH levels were carried out as previously described (13). For transient transfection experiments, the hGH reporter gene was replaced by a firefly luciferase cDNA. Mutations in the AP-1 and Ets binding sites were generated by oligonucleotide-directed mutagenesis and confirmed by dideoxy sequence analysis as previously described (49). Experiments to identify GM-CSF-responsive enhancer sequences were generated from a SEP template as restriction fragments or PCR-amplified products and subcloned in front of the −696 to +46 promoter-luciferase expression vectors.

Cell culture and RNA analysis. Bone marrow progenitor cells were purified and cultured as previously described (13). Recombinant cytokines were obtained from R&D Systems (M- and GM-CSF) and used at the following concentrations unless otherwise noted: human M-CSF, 20 ng/ml; mouse GM-CSF, 5 ng/ml. U-937 and THP-1 cells (both from the American Type Culture Collection) and Ba/F3 parental cells and derivatives (generous gift of A. D. D'Andrea) were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Gemini), IL-3, penicillin (100 U/ml), and streptomycin (100 mg/ml). Total RNA was isolated by the guanidium thiocyanate method (2). RNase protection assays were performed as described previously (26). The antisense RNA probe for the murine SR corresponds to codons 84 to 158 that are common to both type I and II SR. The antisense probe for hGH corresponds to the *Bgl*II/*Sma*I fragment of exon 5 of the hGH gene.

Transient transfection. Cell lines were transiently transfected by electroporation (5, 50) in 200 μl of OptiMEM (Gibco) with 10 μg of luciferase reporter plasmid and 0.1 μg of β-actin-*lacZ* reporter plasmid, as an internal transfection control, at 250 V and 960 μF, using a Bio-Rad electroporator with capacitance extender. Cells were resuspended in RPMI 1640 and incubated with additional GM-CSF (5 ng/ml) for 12 to 72 h. Salmon sperm DNA was used to balance all transfection groups to equal amounts of transfected DNA. Luciferase and β-galactosidase enzymatic activities were determined as previously described (25), and luciferase activity was normalized to the β-galactosidase standard.

Nuclear extract preparation and DNA binding assays. Nuclear extracts were prepared from exponentially growing U-937 and Ba/F3 cell types maintained in the absence or presence of GM-CSF. The cells were then washed with ice-cold phosphate-buffered saline and incubated in buffer A (10 mM HEPES [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol) on ice for 10 min. After centrifugation, the cells were resuspended in buffer A plus 0.2% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 4 mM benzamidine, 10 mg of leupeptin per ml, and 10 mg of aprotinin per ml and placed on ice for 5 to 10 min to allow lysis of the plasma membrane. The nuclei were isolated by centrifugation and resuspended in buffer C (20 mM HEPES [pH 7.9], 20% glycerol, 400 mM KCl, 0.2 mM EDTA, 1 mM dithiothreitol, proteinase inhibitors) and incubated for 30 min at 4°C. After centrifugation at 65,000 rpm for 30 min in a Beckman TLA-100 rotor at 4°C, aliquots of nuclear extract protein were frozen at −80°C.

DNase footprinting studies were performed with 10 to 20 fmol of DNA probe end labeled with ^{32}P to a specific activity of 10,000 cpm/fmol containing 100,000 cpm of radioactivity. The probe was combined with 0 to 30 μg of nuclear extract protein diluted in 20 μl of binding buffer (40 mM KCl, 30 mM HEPES [pH 7.9], 12.5 mM $MgCl_2$, 1 mM dithiothreitol, 20% glycerol, 0.1% Nonidet P-40) as described above and incubated at room temperature for 30 min. Limited DNase digestions were performed at room temperature after addition of 50 μl of a solution containing 10 mM $MgCl_2$ and 5 mM $CaCl_2$ and then 2 to 20 ng of DNase I. After 1 min, the digestion was stopped by addition of 90 μl of a solution containing 20 mM EDTA (pH 8.0), 1% sodium dodecyl sulfate, 0.2 M NaCl, and 125 μg of tRNA per ml. Samples were extracted twice by phenol-chloroform, precipitated, and resolved on a denaturing 10% polyacrylamide gel. The patterns of DNase footprints were visualized by autoradiography.

Electrophoretic mobility shift assays were performed with approximately 1 ng of synthetic double-stranded DNA probes labeled to a specific activity of 100,000 cpm/ng. Probes were combined with 0 to 8.5 μg of nuclear extract protein in 30 μl of total binding buffer [final composition, 5 mM HEPES (pH 7.9), 40 mM KCl, 2.5 mM EDTA, bovine serum albumin (0.15 μg/μl), poly(dI-dC) (0.15 μg/μl)] and incubated on ice for 30 min. Antibodies to specific STAT proteins (Santa Cruz Biotechnology) were added to DNA binding reactions and incubated on ice 20 min prior to electrophoresis. Protein-DNA complexes were resolved by nondenaturing polyacrylamide gel electrophoresis and detected by autoradiography. The sequences of the sense strands of synthetic probes were as follows: footprint B, 5′-GAT CCT GGC TTA AAT TAA TTT TGG ACA CTT TTA CAA CTC ATT TA-3′; footprint C, 5′-GAT CCC TCA TTT AAA CTA CAT GGG TCT TTC CTA CAA TTC TGA AGC CAT AGT TCC CT-3′; and gamma interferon activation sequence (GAS) consensus, 5′-GAT CCA GAT TTC TA GGA ATT CAA TCC A-3′.

RESULTS

M-CSF and GM-CSF regulate SR-A gene expression through distinct enhancers. Expression of the SR-A gene serves as a marker of macrophage differentiation. This gene has previously been demonstrated to contain information in both proximal promoter and distal enhancer elements that target reporter gene expression to macrophages of transgenic mice (13). Within these regions, sites for AP-1, AP-1/Ets ternary complexes, and PU.1, as diagrammed in Fig. 1A, are required for macrophage-specific expression and transcriptional activation of reporter genes in response to phorbol esters (25, 49). To investigate mechanisms responsible for transcriptional activation by M-CSF and GM-CSF, we examined the expression of both the endogenous scavenger receptor gene and SR-A–hGH fusion gene transcripts during differentiation of macrophages from bone marrow progenitor cells. In

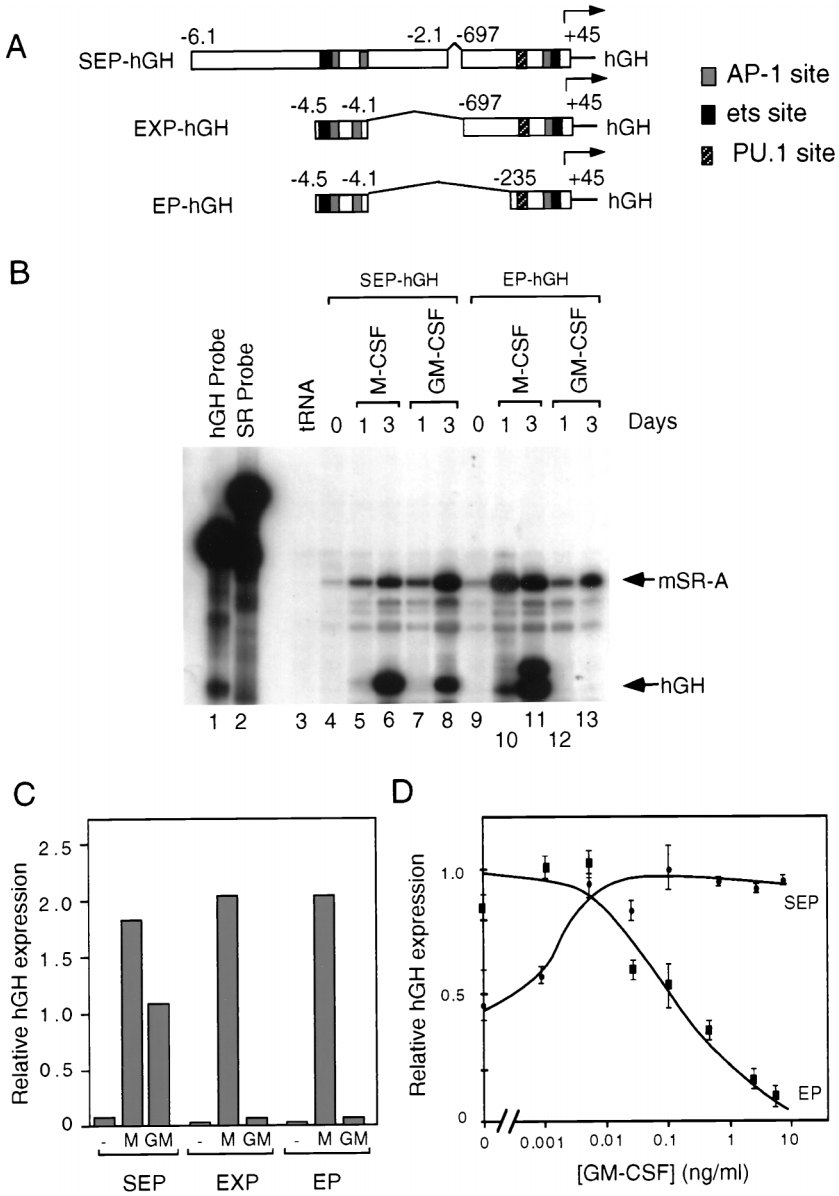

FIG. 1. Differential regulation of SR-A–hGH transgenes by M-CSF and GM-CSF in bone marrow progenitor cells. (A) Schematic diagram of SR-A–hGH transgenes indicating location of PU.1 and AP-1/Ets motifs defined by biochemical and transfection-based studies (25, 49). Each EP construct was linked to an hGH reporter gene as previously described (13). (B) Analysis of SR-A and hGH mRNA levels in bone marrow progenitor cells obtained from SEP-hGH and EP-hGH transgenic mice treated with M-CSF or GM-CSF. RNA levels were determined by simultaneous hybridization of antisense hGH and SR-A cRNA probes with total RNA, followed by cleavage with RNase A. Protected fragments are indicated at the right. In all figures where used, the prefix "m" denotes "murine." (C) Regulation of SR-A–hGH transgenes by M-CSF and GM-CSF as assessed by hGH content. Results are expressed as the ratio of hGH content in progenitor cells to the hGH content in peritoneal macrophages obtained from the same animal. This normalization method permits comparisons between transgenic lines that exhibit quantitative differences in transgene expression due to integration site. (D) Effects of the combination of M-CSF and GM-CSF on SR-A–hGH transgene expression. Bone marrow progenitor cells from the SEP-hGH and EP-hGH transgenic lines were cultured in the presence of 20 ng of M-CSF per ml and the indicated concentrations of GM-CSF. hGH content was determined 72 h after plating. Error bars represent standard error of the mean. The data are representative of experiments analyzing seven founder lines for SEP-hGH, six founder lines for EXP-hGH, and six founder lines for EP-hGH.

an RNase protection assay, bone marrow cells were found to exhibit little or no expression of either endogenous scavenger receptor mRNA or reporter gene expression, whether directed by information extending to −6.5 kb from the transcriptional start site (SEP-hGH [Fig. 1B]) or a minimal EP element (EP-hGH [Fig. 1B, lanes 4 and 9]).

Culture of progenitor cells in the presence of M-CSF to promote macrophage proliferation and differentiation resulted in marked increases in both scavenger receptor mRNA and transgenic reporter (hGH) mRNA directed by either the SEP or EP regulatory element (Fig. 1B, lanes 5, 6, 10, and 11). Thus, the minimal EP regulatory elements were sufficient to confer M-CSF responsiveness, consistent with previous studies (14). Culture of progenitor cells in GM-CSF to promote differentiation of granulocytes and macrophages also resulted in a marked increase in expression of the endogenous scavenger gene in the adherent macrophage population (Fig. 1B, lanes 7, 8, 12, and 13). However, while the longer SEP-hGH transgene

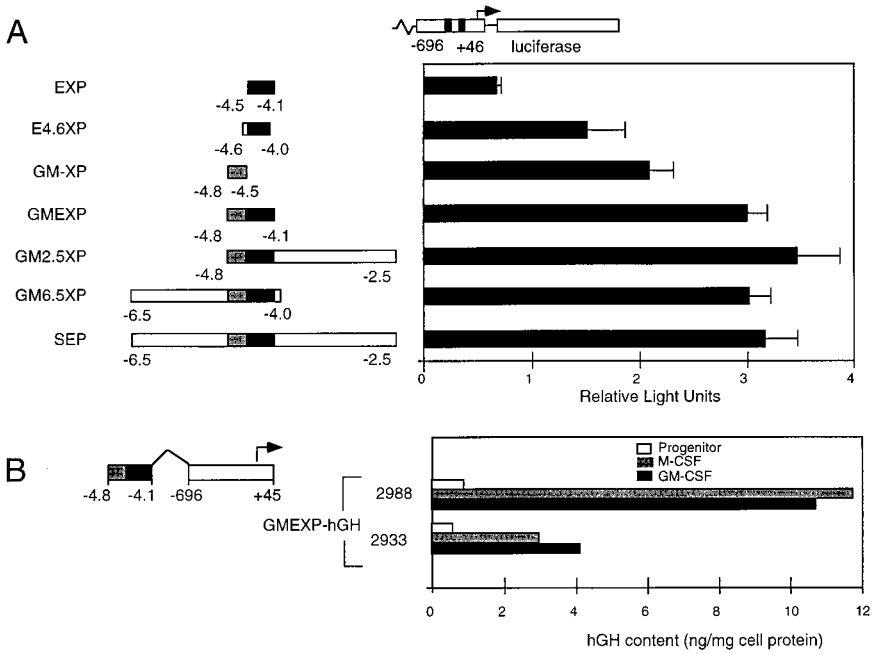

FIG. 2. Definition of a GM-CSF-dependent enhancer. (A) Primary bone marrow progenitor cells were cultured in the presence of GM-CSF for 24 h prior to being electroporated with SR-A–luciferase fusion genes containing the −696 to +46 promoter linked to the indicated upstream region. The M-CSF-dependent enhancer is shown in black. The minimum sequence required for GM-CSF-dependent enhancer activity is shown in grey. Promoter activities are expressed relative to the activity for EXP-luc. The results represent the averages of three independent experiments performed in duplicate ± standard error of the mean. (B) Analysis of GMEXP promoter activity in transgenic mice. The regulatory elements illustrated for GMEXP were linked to hGH and used to generate two transgenic mouse lines. Bone marrow progenitor cells from these lines were plated in the presence of M-CSF or GM-CSF, and hGH content was determined 72 h later. Open bars represent hGH levels in freshly isolated progenitor cells. Each bar represents the mean of duplicate points. The experiment is representative of three independent experiments.

was also responsive to GM-CSF (Fig. 1B, lanes 7 and 8), the minimal EP-hGH transgene was not (Fig. 1B, lanes 12 and 13). The differential responses of the SEP-hGH and EP-hGH transgenes were confirmed by quantitation of reporter (hGH) protein levels in progenitor cells treated for 3 days with either M-CSF or GM-CSF (Fig. 1C). Thus, regulatory elements in the minimal EP of the SR-A gene were sufficient for responsiveness to M-CSF but were not sufficient to mediate a transcriptional response to GM-CSF.

We next evaluated the effects of combinations of M-CSF and GM-CSF on the expression of SEP-hGH and EP-hGH transgenes. Bone marrow progenitor cells were cultured in the presence of 20 ng of M-CSF per ml and increasing concentrations of GM-CSF. As illustrated in Fig. 1D, treatment of cells with the combination of M-CSF and GM-CSF led to a further increase in expression of the SEP-hGH transgene. These factors may act synergistically, because the half-maximal response to the addition of GM-CSF occurred at a concentration that alone has no effect on SEP expression (~0.05 ng/ml) and is insufficient to promote macrophage differentiation (data not shown). Surprisingly, GM-CSF strongly inhibited the response of the minimal EP-hGH transgene to M-CSF. Thus, GM-CSF exerted opposite effects on M-CSF-dependent transcription, dependent on the presence or absence of GM-CSF regulatory elements. These observations were confirmed in assays using several independently derived transgenic lines (data not shown) and therefore do not represent artifacts of the site of integration of the transgene. To determine whether the inability of the minimal EP-hGH transgene to respond to GM-CSF was due to removal of proximal sequences (−697 to −235) or to removal of sequences flanking the distal enhancer, we generated transgenic mice in which the minimal enhancer (−4.1 to −4.5 kb) was linked to promoter information extending from −697 to +45 bp (EXP-hGH [Fig. 1A]). This transgene was responsive to M-CSF but did not respond to GM-CSF (Fig. 1C). These results indicated that sequences required for GM-CSF responsiveness resided in the distal enhancer.

To identify *cis*-active elements responsible for transcriptional activation of the SR gene in response to GM-CSF, a series of deletion mutants of the upstream regulatory elements were evaluated in transfected bone marrow progenitor cells cultured in the presence of GM-CSF. These experiments suggested that regulatory elements necessary for transcriptional activation by GM-CSF resided in a 300-bp region immediately upstream of the M-CSF-dependent enhancer element present in the EXP construct (Fig. 2A). To determine whether these sequences were sufficient to confer GM-CSF-dependent activation to the minimal EP regulatory elements in vivo, two transgenic lines were established. Both of these lines demonstrated GM-CSF-dependent induction of hGH reporter gene expression in bone marrow progenitor cells (Fig. 2B), thus defining the 300-bp region from −4.5 to −4.8 kb as a GM-CSF-responsive enhancer element.

Activation of SR-A gene expression by M-CSF is mediated by AP-1 and cooperating Ets factors. Previous experiments in the THP-1 monocytic leukemia cell line suggested that AP-1 and Ets protein binding sites were necessary for activation of the SR-A gene during macrophage differentiation induced by phorbol ester (50). To evaluate the roles of AP-1 and Ets proteins in regulating SR-A expression in vivo, mutations were introduced into either the three AP-1 sites or the two Ets sites in the context of the EXP-hGH transgene (Fig. 3A). The resulting transgenic lines were first assayed for expression in the peritoneal macrophage population. Mutation of each of the AP-1 sites and each of the Ets sites resulted in measurable hGH expression in peritoneal macrophages in one of six and

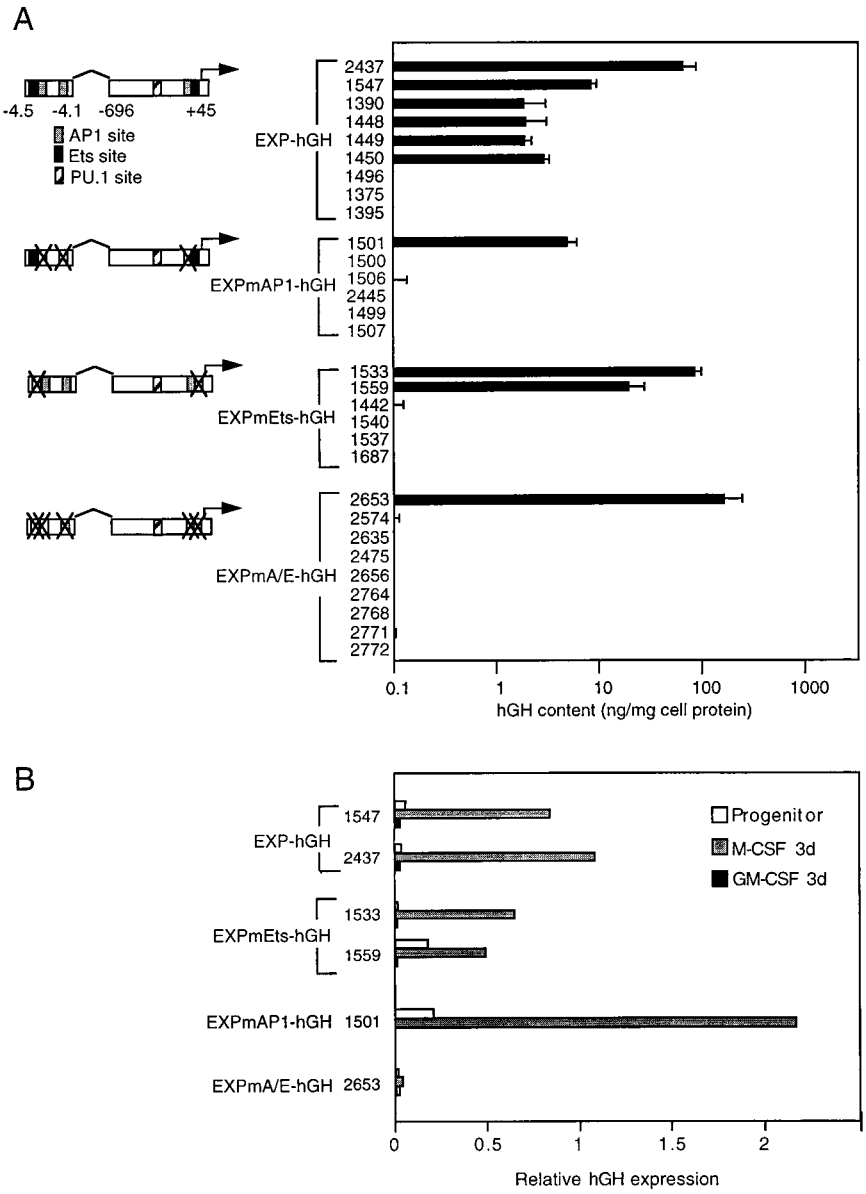

FIG. 3. Role of AP-1/Ets motifs in mediating the responses of SR-A to M-CSF. Point mutations were introduced into each of the AP-1 sites (EXPmAP-1-hGH), each of the adjacent Ets sites (EXPmEts-hGH), or both the AP-1 and Ets sites (EXPmA/E-hGH) in the context of the EXP-hGH transgene. Each construct was used to generate several founder animals which were bred to establish pedigrees (independently derived transgenic lines are indicated by the numbers to the left of the bar graph). (A) Expression of hGH in peritoneal macrophages obtained from each pedigree. (B) Responses of wild-type and mutant EXP transgenes to M-CSF and GM-CSF. Bone marrow progenitor cells were obtained from transgenic lines exhibiting macrophage expression and cultured in the presence of M-CSF or GM-CSF for 72 h (3 days [3d]) prior to measurement of hGH content.

two of six independently derived transgenic lines, respectively, while the control EXP transgene was expressed in six of nine independently derived lines. Although the differences in the percentage of expression observed for the mutant SR-A promoters and the wild-type promoter did not reach statistical significance (EPmAP1-hGH versus EXP-hGH, $P = 0.1477$; EPmets-hGH versus EXP-hGH, $P = 0.6889$, using two tailed P values adjusted for multiple comparisons), we noted that only high-copy-number (10 to 100) mutant promoter constructs expressed hGH, whereas even single-copy integrations of the wild-type EP-hGH construct expressed hGH. To determine whether transgenes containing mutations in AP-1 or Ets sites were regulated by M-CSF, experiments were performed in bone marrow progenitor cells. Transgenic lines that exhibited no reporter gene expression in peritoneal macrophages also exhibited no expression in M-CSF-treated bone marrow progenitor cells (data not shown). Each of the transgenes containing selective mutations in AP-1 or Ets binding sites that were expressed in peritoneal macrophages was also found to be activated in progenitor cells in response to M-CSF (Fig. 3B). We therefore next evaluated the expression of transgenes in which the AP-1 and Ets sites were mutated in combination (EXPmAE-hGH). Only one of nine independently derived founder animals was found to be expressed in peritoneal macrophages, which represented a statistically significant difference in penetrance compared to the EXP transgene ($P =$

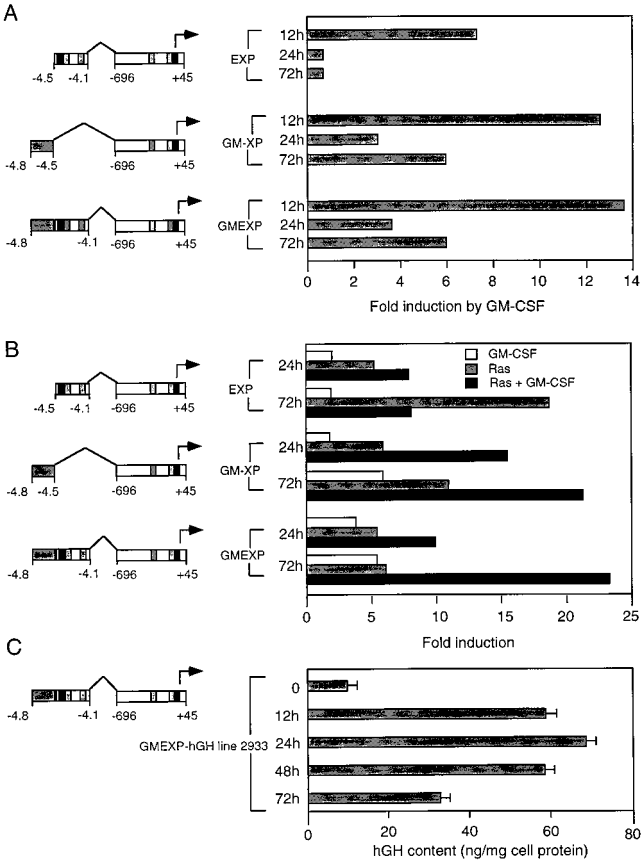

FIG. 4. Responses of SR regulatory elements to GM-CSF and activated Ras in Ba/F3-GMR cells and transgenic mice. (A) GM-CSF responsiveness of SR regulatory elements in Ba/F3-GMR cells. Cells were transfected with the indicated SR constructs and treated with GM-CSF or vehicle. Luciferase activity was determined 12, 24, or 72 h later. (B) Responsiveness of SR regulatory elements to the combination of GM-CSF and activated Ras. Ba/F3-GM cells were transfected with the indicated SR constructs and an expression plasmid directing the expression of a constitutively active form of Ras (Val^{12}-H-Ras) as indicated. Cells were treated with GM-CSF or vehicle, and luciferase activity was determined 24 or 72 h later. (C) Immediate responses of the GMEXP-hGH transgene to GM-CSF in bone marrow-derived macrophages. Bone marrow progenitor cells were harvested from GMEXP-hGH transgenic line 2933 and cultured for 48 h in M-CSF to obtain a homogeneous population of adherent macrophages. The cells were then treated with GM-CSF and harvested for analysis of hGH activity 12, 24, 48, or 72 h later.

0.045). Furthermore, the one transgenic line that was found to be expressed in peritoneal macrophages exhibited no response to M-CSF in bone marrow progenitor cells. In concert, these results indicate that AP-1 and cooperating Ets domain transcription factors are required for the transcriptional responses of the SR-A gene to M-CSF.

Differential regulation of SR-A enhancers by GM-CSF and Val^{12}-Ras. Because previous studies have demonstrated that GM-CSF can activate AP-1 and Ets-dependent promoters, we wished to further explore the basis for the differential effects of GM-CSF and M-CSF on SR-A transgenes that were observed in bone marrow progenitor cells. Experiments were performed in Ba/F3-GMR cells, which were derived from an IL-3-dependent B-cell leukemia by stable transfection of an expression vector encoding the α subunit of the GM-CSF receptor (50). In contrast to the parental Ba/F3 cells, Ba/F3-GMR cells confer transcriptional responses to GM-CSF. As illustrated in Fig. 4A, GM-CSF not only stimulated transcription from promoters containing the GM-CSF-responsive enhancer (GM-XP-luc and GMEXP-luc) but also activated the M-CSF-responsive promoter (EXP-luc). However, the durations of the transcriptional responses of the two classes of promoters were significantly different. The response of the M-CSF-responsive promoter, EXP-luc, was very transient, being maximal 12 h after GM-CSF treatment and returning to basal levels by 24 h. Induction of EXP-luc by GM-CSF was dependent on the AP-1 and Ets binding motifs, because point mutations of either of these classes of binding sites abolished the GM-CSF response (data not shown). In contrast, reporter genes containing the GM-CSF-responsive enhancer element defined in vivo (GM-XP-luc and GMEXP-luc) continued to exhibit high levels of expression for as long as 72 h following treatment with GM-CSF.

We next evaluated the effects of combinations of GM-CSF and independent activation of the Ras signaling pathway on M-CSF- and GM-CSF-responsive promoters in Ba/F3-GMR cells. Reporter gene expression was evaluated at 24 and 72 h, by which time GM-CSF activation of AP-1 and Ets proteins is no longer measurable. Cotransfection of the Val^{12}-Ras vector resulted in a sustained increase in expression of each of the luciferase reporter genes (Fig. 4B), indicating that activation of the Ras pathway is alone sufficient to activate the M-CSF-responsive enhancer in these cells. Val^{12}-Ras also activated GM-XP, suggesting that the GM-CSF-responsive enhancer is also activated by Ras, as the −696 to +45 promoter is relatively unresponsive by itself in this assay (reference 49 and data not shown). When cells were cotransfected with Val^{12}-Ras and treated with GM-CSF, additive or slightly more than additive effects were observed for the GM-XP-luc and GMEXP-luc reporter genes. In contrast, the combination of GM-CSF and cotransfected Val^{12}-Ras resulted in less activity of the EXP-luc reporter gene at 72 h than was observed in the presence of cotransfected Val^{12}-Ras alone. Thus, at extended time points, GM-CSF inhibited, rather than potentiated, Ras-dependent activation of the M-CSF-dependent enhancer, similar to the effects of GM-CSF on M-CSF-dependent expression of EP-hGH and EXP-hGH observed in transgenic bone marrow progenitor cells.

Although an immediate-early response of transgenes containing the GM-CSF-dependent enhancer to GM-CSF was not observed in undifferentiated bone marrow progenitor cells (Fig. 1B), macrophage precursor cells represent a very small percentage (<3%) of this cell population. To determine whether the GM-CSF enhancer can also confer an immediate-early response to GM-CSF in macrophages, bone marrow progenitor cells were harvested from GMEXP-hGH transgenic mice and cultured in M-CSF for 48 h to establish a homogeneous population of adherent macrophages. The cells were then treated with GM-CSF for 12, 24, 48, and 72 h and harvested for analysis of hGH content. These experiments demonstrated a significant increase in hGH expression as early as 12 h following GM-CSF treatment, consistent with the immediate-early response observed in Ba/F3-GMR cells (Fig. 4C).

The GM-CSF enhancer is recognized by both constitutively expressed and inducible DNA binding proteins. The functional characteristics of the GM-CSF-responsive enhancer suggested that it should be recognized by proteins accounting for both immediate and sustained transcriptional responses to GM-CSF. DNase I footprinting experiments were therefore performed with nuclear proteins extracted from Ba/F3-GMR cells at various times following treatment with GM-CSF. As illustrated in Fig. 5A, footprinting analysis of the sense and antisense strands revealed several hypersensitive sites and the presence of five footprints, labeled A through E, that encom-

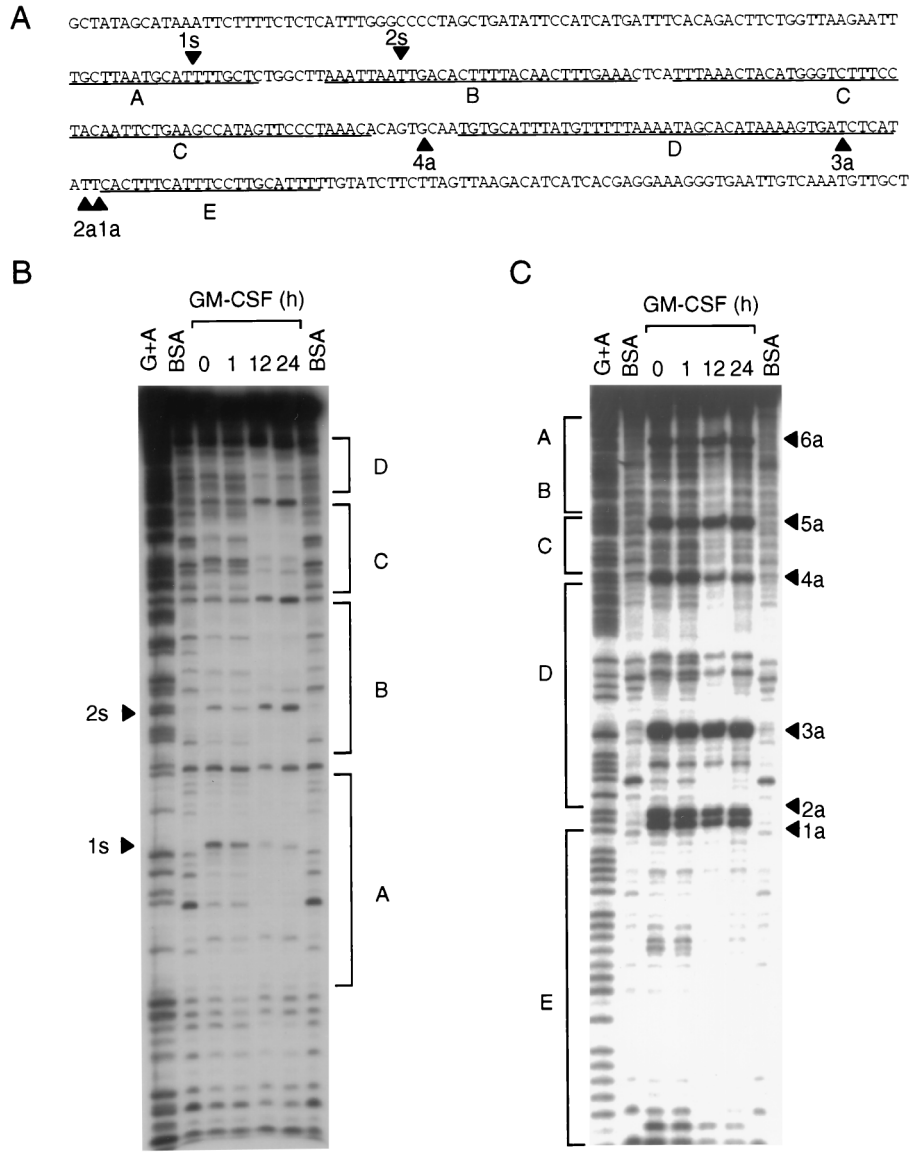

FIG. 5. Interaction of GM-CSF-inducible DNA binding activities with the SR GM-CSF-responsive enhancer. (A) Sequence of the GM-CSF-responsive enhancer. Footprints in panels B and C are indicated by underlines. Hypersensitive sites in the sense strand are indicated as 1s and 2s. Hypersensitive sites in the antisense strand are indicated as 1a, 2a, etc. (B) DNase I footprint analysis of the sense strand of the GM-CSF-responsive enhancer, using nuclear extracts prepared from control Ba/F3 GMR cells and Ba/F3 cells treated with GM-CSF for 1, 12, and 24 h. BSA, bovine serum albumin. (C) DNase I footprint analysis of the antisense strand of the GM-CSF-responsive enhancer, using the same nuclear extracts described for panel B.

passed nearly the entire GM-CSF-dependent enhancer. There was no significant change in the footprint pattern in nuclear extracts prepared from cells treated with GM-CSF for 1 h. However, by 12 h of GM-CSF treatment, occupancy of footprint C was significantly increased, the extents of footprints A, B, D, and E had expanded, and an altered profile of hypersensitive sites was apparent. These footprints remained mostly unchanged after 24 h of GM-CSF treatment, although a slight decrease in footprints D and C was noted. Thus, these experiments were consistent with a class of proteins that were constitutively bound and could potentially mediate immediate responses to GM-CSF, as well as a second class of proteins induced by GM-CSF that either replace or complement the constitutively bound proteins and contribute to the delayed transcriptional responses.

To investigate the possible identities of the factors binding to the GM-CSF-responsive enhancer, we performed a series of electrophoretic mobility shift assays using synthetic DNA probes corresponding to specific footprints. Representative experiments obtained for probes corresponding to footprints B and C are illustrated in Fig. 6. Nuclear extracts prepared from untreated Ba/F3-GMR cells contained DNA binding activities for both of these probes that were not altered by treatment with GM-CSF for 30 min, consistent with the results of DNase I footprinting experiments. Similar results were obtained with DNA probes corresponding to footprints A and D (data not shown). In contrast, treatment of Ba/F3-GMR cells with GM-CSF for 30 min strongly induced a DNA binding activity for an oligonucleotide containing a consensus GAS element that could be partially supershifted by an antibody directed against

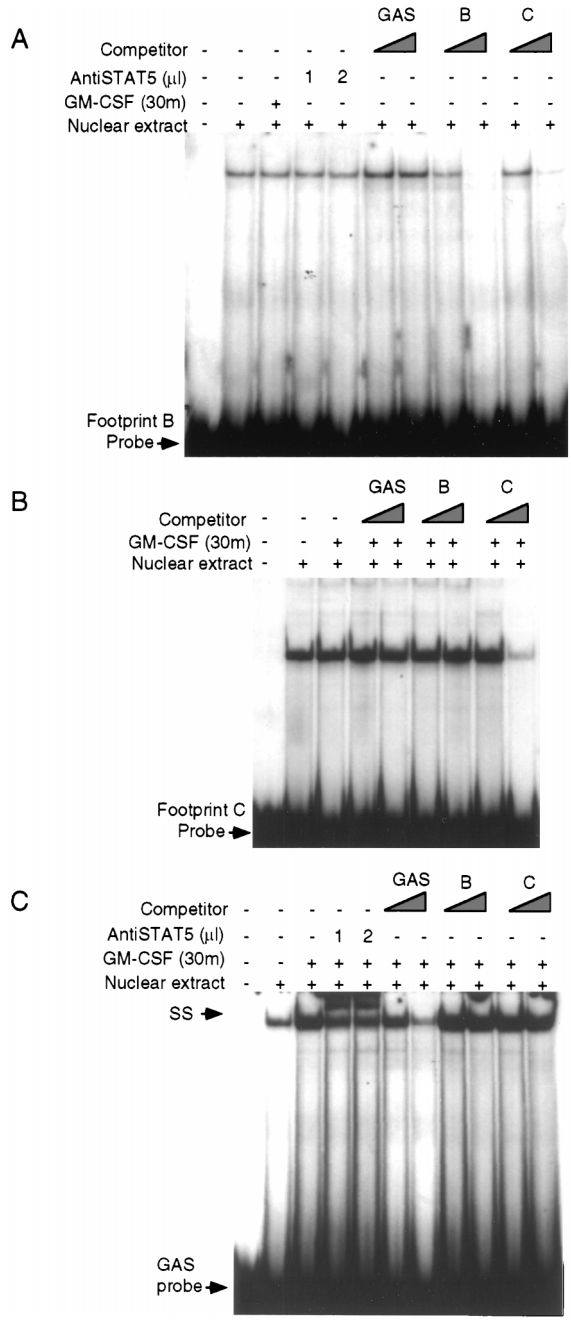

FIG. 6. Constitutively expressed proteins that bind to the GM-CSF-responsive promoter are distinct from GAS binding proteins. Nuclear proteins were isolated from untreated Ba/F3-GMR cells or Ba/F3-GMR cells treated for 30 min with GM-CSF as indicated. (A) Binding of Ba/F3-GMR nuclear proteins to a ^{32}P-labeled footprint B probe. Triangles represent the addition of 10 or 100 ng of unlabeled competitor oligonucleotides containing a consensus GAS element, footprint B, or footprint C. (B) Binding of Ba/F3-GMR nuclear proteins to a ^{32}P-labeled footprint C probe. Triangles represent the competing oligonucleotides described for panel A. (C) Binding of nuclear proteins from Ba/F3-GMR cells to a GAS oligonucleotide probe. A supershifted complex observed in the presence of antibody that recognizes STAT5a and STAT5b is indicated by SS. Triangles represent competing antibodies as described for panel A.

STAT5a and STAT5b (Fig. 6C). The GAS element failed to compete the binding of proteins to the footprint B and C oligonucleotides, and the antibody that recognizes STAT5a and STAT5b had no effect on the binding of these factors (Fig. 6A and B). Anti-STAT1 antibodies also had no influence on these DNA binding activities (data not shown). Thus, the factors that bind to footprints B and C of the GM-CSF-dependent enhancers are unlikely to be STAT proteins that are capable of recognizing the consensus GAS element, and they are immunologically distinct from STAT5a/b and STAT1. The protein DNA complexes forming on the footprint B and C oligonucleotides exhibited significantly different electrophoretic mobilities, and the footprint B oligonucleotide had no effect on the binding of proteins to footprint C. At high concentrations, the footprint C oligonucleotide reduced binding of proteins to footprint B, suggesting that it contains a weak binding site for the footprint B DNA binding activity. In concert, these experiments suggest that the GM-CSF-dependent enhancer is recognized by several distinct factors that together mediate both immediate and delayed responses to GM-CSF. The specific identities of these DNA binding proteins remain to be determined.

Inhibition of the GM-CSF-responsive enhancer by dominant negative forms of JAK2 and STAT5. The finding that STAT5 proteins did not bind directly to the GM-CSF enhancer raised the question of whether JAK2 and/or STAT5 was required for transactivation. To address this question, we initially evaluated the function of the GM-CSF enhancer in Ba/F3 cells that stably express either the wild-type human common β subunit of the GM-CSF/IL-3/IL-5 receptor or C-terminal deletion mutants. Transactivation of the β subunit at residue 544 abolishes activation of the Ras pathway, while further transactivation to residue 455 prevents association of JAK2. As illustrated in Fig. 7A, truncation of the β subunit at residue 544 almost completely abolished the transcriptional response of the GM-CSF-responsive promoter to GM-CSF treatment, consistent with the ability of constitutively active Ras to activate this promoter.

We next evaluated effects of wild-type and dominant negative forms of STAT5 and JAK2 (16a) on the transcriptional activity of the GM-CSF-dependent promoter GM-XP-luc in Ba/F3 cells expressing the murine GM-CSFα subunit. As illustrated in Fig. 7B, overexpression of STAT5a and JAK2, but not STAT1α, potentiated the activity of the GM-CSF-dependent enhancer at 24 h, while dominant negative forms of STAT5 and JAK2 strongly inhibited transcription. Similar effects were observed at 72 h (data not shown). In contrast, dominant negative STAT5 did not inhibit Ras-dependent activation of a promoter containing the scavenger receptor AP-1/Ets sites (Fig. 7C).

DISCUSSION

Cells of the macrophage lineage serve diverse homeostatic and immunologic functions by differentiating into functionally distinct end cells that proliferate and differentiate under the influence of M-CSF, GM-CSF and other locally produced regulatory molecules (10). Studies of the osteopetrotic *op*/*op* mouse indicate that the macrophage lineage can be considered to consist of M-CSF-dependent and M-CSF-independent populations (47, 48). Mice homozygous for the *op* mutation lack M-CSF (46) and exhibit striking defects in some but not all macrophage populations. Osteoclasts, peritoneal macrophages, and lymph node subcapsular macrophages, for example, are severely reduced or absent. Other populations, however, including phagocytes in the splenic red pulp, thymic cortex, Kupffer cells, alveolar macrophages, and microglia, are largely unaffected in the absence of M-CSF (47, 48). These observations indicate the presence of multiple pathways for

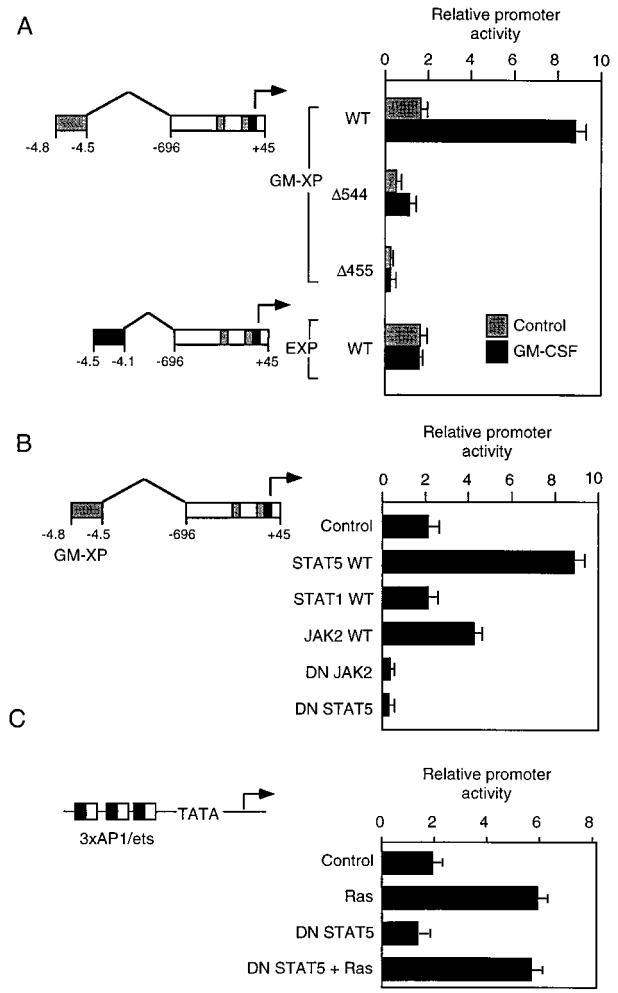

FIG. 7. Roles of Ras, JAK2, and STAT5 in regulation of the GM-CSF-responsive enhancer. (A) Deletion of the distal C terminus of the common β subunit of the GM-CSF/IL-3/IL-5 receptor abolishes GM-CSF responsiveness of the enhancer. Ba/F3 cells that stably express the human β subunit of the GM-CSF/IL-3/IL-5 receptor or the indicated C-terminal deletion mutants were transfected with the GM-XP luciferase reporter gene, treated with human GM-CSF, and harvested for analysis of luciferase activity 24 h later. (B) Dominant negative forms of JAK2 and STAT5 block transcriptional activation of the GM-CSF-responsive enhancer. Ba/F3-GMR cells were transfected with the GM-XP-luc reporter gene and plasmids directing the expression of STAT5a, STAT1α, JAK2, dominant negative JAK2, or dominant negative STAT5, treated with GM-CSF, and harvested for analysis of luciferase activity 24 h later. (C) Dominant negative STAT5 does not inhibit activation of a promoter under transcriptional control of the scavenger receptor AP-1/Ets sites by Val^{12}-H-Ras. Ba/F3-GMR cells were transfected with a luciferase reporter gene in which three copies of the SR-A M-CSF enhancer AP-1/Ets sites were linked to a minimal promoter. Cells were cotransfected with Val^{12}-H-Ras and or dominant negative STAT5 expression plasmids as indicated and harvested for analysis of luciferase activity 48 h later.

macrophage differentiation that give rise to cells that have overlapping but nonidentical functions.

The CSFs responsible for M-CSF-independent macrophage populations appear to be highly redundant. Disruptions of the genes encoding GM-CSF or the common β subunit of the GM-CSF, IL-3, and IL-5 receptors have had relatively little effect on any of the macrophage lineages, with the exception of a functional defect in the ability of alveolar macrophages to clear surfactant components (8, 29, 30). Thus, GM-CSF is not essential for the development of M-CSF-independent populations of macrophages. Nevertheless, the potent activities of GM-CSF in promoting the development of macrophage colonies in vitro and its expression by many cell types in vivo support the idea that it plays important physiologic roles in establishing M-CSF-independent macrophage populations.

Although M-CSF and GM-CSF promote different sets of specialized functions, many of the phenotypic characteristics of M-CSF- and GM-CSF-derived macrophages are similar. At the gene level, M-CSF and GM-CSF induce the expression of many macrophage- or myeloid-specific genes in parallel, including the SR-A gene. Because the receptors for M-CSF and GM-CSF are not related, these observations have raised the question of whether coordinate regulation of these genes reflects (i) activation of distinct sets of transcription factors that function independently or (ii) convergence of signal transduction pathways on a common set of transcription factors.

In this study, we have examined the mechanisms by which M-CSF and GM-CSF coordinately regulate the expression of the macrophage SR-A gene. SR regulatory elements consisting of a minimal 245-bp promoter and a 400-bp upstream enhancer were sufficient to confer macrophage-specific expression and M-CSF-dependent regulation in transgenic mice. Several lines of evidence indicate that transcriptional activation of the SR-A gene by M-CSF is mediated by a Ras-dependent signal transduction cascade that stimulates the expression and activities of AP-1 and Ets domain transcription factors. We have previously shown that c-Jun, JunB, and Ets2 bind to regulatory elements in the M-CSF-dependent enhancer and act synergistically to stimulate transcription (49). In the present study, we have shown that mutation of these binding sites abolishes SR-A promoter responses to M-CSF in transgenic mice, while expression of a constitutive form of Ras is sufficient to activate the M-CSF-responsive enhancer.

These observations raised the possibility that GM-CSF acts to regulate the SR-A gene in a similar manner, as the GM-CSF receptor also couples to Ras-dependent signal transduction pathways and activates AP-1 and Ets transcription factors (1, 16, 37, 50). Consistent with this possibility, GM-CSF was indeed capable of activating the M-CSF-responsive enhancer in Ba/F3-GMR cells, and this effect was mediated by AP-1 and Ets transcription factors. However, the response of the M-CSF-dependent enhancer to GM-CSF was very transient in Ba/F3 cells, corresponding to the temporal profile of an immediate-early gene. Furthermore, when evaluated in transgenic mice, GM-CSF not only was unable to activate SR-A promoter activity through the M-CSF-responsive enhancer but inhibited activation by M-CSF.

Regulation of the SR-A gene by GM-CSF in transgenic mice was instead found to depend on a distinct GM-CSF-responsive enhancer. This enhancer could be activated by both Ras and GM-CSF in a cell line expressing functional GM-CSF receptors, with maximal activation observed 72 h following GM-CSF treatment. DNase I footprinting experiments of the GM-CSF-responsive promoter using nuclear extracts prepared from these cells indicated the presence of both constitutively expressed DNA binding proteins, as well as GM-CSF-inducible proteins that become maximally expressed 12 h following GM-CSF treatment. The identities of these proteins remain to be established, but they do not appear to correspond to known STAT factors. The constitutively expressed proteins are likely to be subject to posttranslational regulation by Ras-dependent signal transduction pathways, because the GM-CSF-responsive enhancer exhibited relatively little transcriptional activity in Ba/F3-GMR resting cells in which these proteins were found to be present but was very active following forced expression of constitutively active Ras. Similarly, truncation of the distal C terminus of the GM-CSF/IL-3/IL-5 receptor β subunit, which

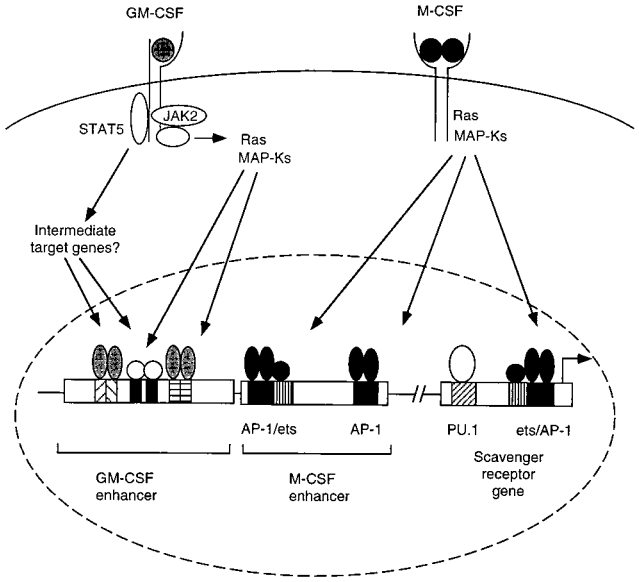

FIG. 8. Mechanisms of transcriptional control of the SR-A gene by M-CSF and GM-CSF. Activation of the SR-A gene by M-CSF is mediated by a M-CSF-dependent enhancer that is recognized by AP-1 and cooperating Ets domain transcription factors. These transcription factors are downstream targets of a Ras-dependent mitogen-activated protein kinase (MAP-K) cascade that controls both their levels of expression and their transcriptional activities. Activation of the SR-A gene by GM-CSF is dependent on a distinct enhancer element located immediately upstream of the M-CSF-dependent enhancer. The GM-CSF-dependent enhancer is proposed to be occupied by constitutively expressed transcription factors that are regulated posttranslationally by Ras and which confer immediate-early transcriptional responses. Sustained expression of the SR-A gene in response to GM-CSF may be mediated in part by an additional set of transcription factors that are direct targets of STAT5 proteins.

couples to Ras, significantly reduced GM-CSF-dependent transcription. While immediate-early effects of Ras on the GM-CSF-dependent enhancer are likely mediated by intracellular signaling pathways, delayed effects could potentially reflect the induction of other cytokines that indirectly activate the GM-CSF-dependent promoter in an autocrine manner.

In concert, our findings are most consistent with a model in which M-CSF and GM-CSF regulate the expression of the SR-A gene by fundamentally different mechanisms (Fig. 8). While Ras appears to play an important role in regulating both enhancers, cross talk between GM-CSF-dependent signal transduction pathways and targets of the M-CSF receptor that are activated by Ras is insufficient to mediate activation of the M-CSF-responsive enhancer in primary bone marrow progenitor cells. The relatively restricted utilization of Ras signaling pathways by M-CSF and GM-CSF may reflect the differential assembly of receptor-associated signaling complexes that are relatively dedicated to specific downstream targets (43). Differential utilization of Ras signal transduction pathways by M-CSF and GM-CSF is consistent with the distinct phenotypic properties of M-CSF- and GM-CSF-derived macrophages. It will be of considerable interest to identify the transcription factors responsible for activation of the SR-A gene by GM-CSF, as these factors are likely to be important in establishing the phenotypic properties of the M-CSF-independent population of macrophages.

ACKNOWLEDGMENTS

We thank Tanya Schneiderman for assistance with preparation of the manuscript. We also thank Alan D'Andrea for dominant-negative STAT5 and JAK2 expression vectors and helpful discussions.

A.C.L. is supported by a Physician Scientist grant from the NIH. J.S.W. is supported by an NIH Medical Scientist Training Program grant to U.C. San Diego. C.K.G. is an Established Investigator of the American Heart Association. This study was supported by NIH grants CA 52599 and P50HL14197 to C.K.G.

REFERENCES

1. **Adunyah, S. E., T. M. Unlap, F. Wagner, and A. S. Kraft.** 1991. Regulation of c-jun expression and AP-1 enhancer activity by granulocyte-macrophage colony stimulating factor. J. Biol. Chem. **266:**5670–5675.
2. **Ausubel, F., R. Brent, R. Kingston, D. Moore, J. Seidman, J. Smith, and K. Struhl (ed.).** 1990. Current protocols in molecular biology. John Wiley & Sons, New York, N.Y.
3. **Brizzi, M. F., M. G. Aronica, A. Rosso, G. P. Bagnara, Y. Yarden, and L. Pegoraro.** 1996. Granulocyte-macrophage colony-stimulating factor stimulates JAK2 signaling pathway and rapidly activates p93fes, STAT1 p91, and STAT3 p92 in polymorphonuclear leukocytes. J. Biol. Chem. **271:**3562–3567.
4. **Brizzi, M. F., M. G. Zini, M. G. Aronica, J. M. Blechman, Y. Yarden, and L. Pegoraro.** 1994. Convergence of signaling by interleukin-3, granulocyte-macrophage colony-stimulating factor, and mast cell growth factor on JAK2 tyrosine kinase. J. Biol. Chem. **269:**31680–31684.
5. **Chen, C. A., and H. Okayama.** 1988. Calcium phosphate-mediated gene transfer: a highly efficient transfection system for stably transforming cells with plasmid DNA. BioTechniques **6:**632–640.
6. **Das, S. K., E. R. Stanley, L. J. Guilbert, and L. W. Forman.** 1981. Human colony-stimulating factor (CSF-1) radioimmunoassay: resolution of three subclasses of human colony-stimulating factors. Blood **58:**630–641.
7. **de Villiers, W. J. S., I. Fraser, D. Hughes, A. G. Doyle, and S. Gordon.** 1994. Macrophage-colony-stimulating factor selectively enhances macrophage scavenger receptor expression and function. J. Exp. Med. **180:**705–709.
8. **Dranhoff, G., A. D. Crawford, M. Sadelain, B. Ream, A. Rashid, R. T. Bronson, G. R. Dickersin, C. J. Bachurski, E. L. Mark, J. A. Whitsett, and R. C. Mulligan.** 1994. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science **264:**713–716.
9. **Geng, Y. J., T. Kodama, and G. K. Hansson.** 1994. Differential expression of scavenger receptor isoforms during monocyte-macrophage differentiation and foam cell formation. Arterioscler. Thromb. **14:**798–806.
10. **Gordon, S.** 1995. The macrophage. Bioessays **17:**977–986.
11. **Heldin, C.-H.** 1995. Dimerization of cell surface receptors in signal transduction. Cell **80:**213–223.
12. **Hill, C. S., and R. Treisman.** 1995. Transcriptional regulation by extracellular signals: mechanisms and specificity. Cell **80:**199–211.
13. **Horvai, A. E., W. Palinski, H. Wu, K. S. Moulton, K. Kalla, and C. K. Glass.** 1995. Scavenger receptor A gene regulatory elements target gene expression to macrophages and to foam cells of atherosclerotic lesions. Proc. Natl. Acad. Sci. USA **92:**5391–5395.
14. **Horvai, A. E., L. Xu, E. Korzus, G. Brard, D. Kalafus, T.-M. Mullen, D. W. Rose, M. G. Rosenfeld, and C. K. Glass.** 1997. Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. Proc. Natl. Acad. Sci. USA **94:**1074–1079.
15. **Ihle, J. N.** 1995. Cytokine receptor signalling. Nature **377:**591–594.
16. **Itoh, T., A. Muto, S. Watanabe, A. Miyajima, T. Yokota, and K.-I. Arai.** 1996. Granulocyte-macrophage colony-stimulating factor provokes RAS activation and transcription of c-fos through different modes of signaling. J. Biol. Chem. **271:**7587–7592.

16a. **Jaster, R., Y. Zhu, M. Pless, S. Bhattacharya, B. Mathey-Prevot, and A. D. D'Andrea.** 1997. JAK2 is required for the induction of the murine *DUB-1* gene. Mol. Cell. Biol. **17:**3664–3672.

17. **Jin, D. I., S. B. Jameson, M. A. Reddy, D. Schenkman, and M. C. Ostrowski.** 1995. Alterations in differentiation and behavior of monocytic phagocytes in transgenic mice that express dominant suppressors of ras signaling. Mol. Cell. Biol. **15:**693–703.
18. **Karin, M.** 1995. The regulation of AP-1 activity by mitogen-activated protein kinases. J. Biol. Chem. **270:**16483–16486.
19. **Kodama, T., M. Freeman, L. Rohrer, J. Zabrecky, P. Matsudaira, and M. Krieger.** 1990. Type I macrophage scavenger receptor contains alpha-helical and X collagen-like coiled coils. Nature **343:**531–535.
20. **Krieger, M., and J. Herz.** 1994. Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). Annu. Rev. Biochem. **63:**601–637.
21. **Lioubin, M. N., G. M. Myles, K. Carlberg, D. Bowtell, and L. R. Rohrschneider.** 1994. Shc, Grb2, Sos1, and a 150-kilodalton tyrosine-phosphorylated protein form complexes with Fms in hematopoietic cells. Mol. Cell. Biol. **14:**5682–5691.
22. **Metcalf, D.** 1988. The molecular control of blood cells. Harvard University Press, Cambridge, Mass.
23. **Metcalf, D.** 1989. The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. Nature **339:**27–30.
24. **Miyajima, A., L.-F. Mui, T. Ogorochi, and K. Sakamaki.** 1993. Receptors for granulocyte and macrophage colony stimulating factor, interleukin 3 and interleukin 5. Blood **82:**1960–1974.
25. **Moulton, K. S., K. Semple, H. Wu, and C. K. Glass.** 1994. Cell-specific

expression of the macrophage scavenger receptor gene is dependent on PU.1 and a composite AP-1/ets motif. Mol. Cell. Biol. **14:**4408–4418.
26. **Moulton, K. S., H. Wu, J. Barnett, S. Parthasarathy, and C. K. Glass.** 1992. Regulated expression of the acetylated LDL receptor gene and isolation of promoter sequences. Proc. Natl. Acad. Sci. USA **89:**8102–8106.
27. **Mui, A. L., H. Wakao, A. M. O'Farrell, N. Harada, and A. Miyajima.** 1995. Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. EMBO J. **14:**1166–1175.
28. **Naito, M., T. Kodama, A. Matsumoto, T. Doi, and K. Takahashi.** 1991. Tissue distribution, intracellular localization, and in vitro expression of bovine macrophage scavenger receptors. Am. J. Pathol. **139:**1411–1423.
29. **Nishinakamura, R., A. Miyajima, P. J. Mee, V. L. Tybulewicz, and R. Murray.** 1996. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. Blood **88:**2458–2464.
30. **Nishinakamura, R., N. Nakayama, Y. Hirabayashi, T. Inoue, D. Aud, T. McNeil, S. Azuma, S. Yoshida, Y. Toyoda, and K. Arai.** 1995. Mice deficient for the IL-3/GM-CSF/IL-5 beta c receptor exhibit lung pathology and impaired immune response, while beta IL3 receptor-deficient mice are normal. Immunity **2:**211–222.
31. **Pawson, T., and G. D. Gish.** 1992. SH2 and SH3 domains: from structure to function. Cell **71:**359–362.
32. **Quelle, F. W., N. Sato, B. A. Witthuhn, R. C. Inhorn, M. Eder, A. Miyajima, J. D. Griffin, and J. N. Ihle.** 1994. JAK2 associates with the βc chain of the receptor for granulocyte-macrophage colony-stimulating factor, and its activation requires the membrane-proximal region. Mol. Cell. Biol. **14:**4335–4341.
33. **Rajotte, D., H. B. Sadowski, A. Haman, K. Gopalbhai, S. Meloche, L. Liu, G. Krystal, and T. Hoang.** 1996. Contribution of both STAT and SRF/TCF to c-fos promoter activation by granulocyte-macrophage colony-stimulating factor. Blood **88:**2906–2916.
34. **Reedijk, M., X. Liu, P. van der Geer, K. Letwin, M. D. Waterfield, T. Hunter, and T. Pawson.** 1992. Tyr721 regulates specific binding of the CSF-1 receptor kinase insert to PI 3′-kinase SH2 domains: a model for SH2-mediated receptor-target interactions. EMBO J. **11:**1365–1372.
35. **Rosen, R. L., K. D. Winestock, G. Chen, X. Liu, L. Hennighausen, and D. S. Finbloom.** 1996. Granulocyte-macrophage colony-stimulating factor preferentially activates the 94-kD STAT5A and an 80-kD STAT5A isoform in human peripheral blood monocytes. Blood **88:**1206–1214.
36. **Roussel, M. F.** 1994. Signal transduction by the macrophage-colony-stimulating factor receptor (CSF-1R). J. Cell Sci. **18:**105–108.
37. **Sato, N., K. Sakamaki, N. Terada, K. I. Arai, and A. Miyajima.** 1993. Signal transduction by high-affinity GM-CSF receptor: two distinct cytoplasmic regions of the common beta subunit responsible for different signaling. EMBO J. **12:**4181–4189.
38. **Sherr, C. J.** 1990. Regulation of mononuclear phagocyte proliferation by colony-stimulating factor 1. Int. J. Cell Cloning **8:**46–62.
39. **Sherr, C. J., C. W. Rettenmier, R. Sacca, M. F. Roussel, A. T. Look, and E. R. Stanley.** 1985. The c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell **41:**665–676.
40. **Stanley, E., and P. M. Heard.** 1977. Factors regulating macrophage production and growth. Purification and some properties of the colony stimulating factor from medium conditioned by mouse L cells. J. Biol. Chem. **252:**4305–4312.
41. **Suzuki, H., Y. Kurihara, M. Takeya, N. Kamada, M. Kataoka, K. Jishage, O. Ueda, H. Sakaguchi, T. Higashi, T. Suzuki, Y. Takashima, Y. Kawabe, O. Cynshi, Y. Wada, M. Honda, H. Kurihara, H. Aburantani, T. Doi, A. Matsumoto, S. Azuma, T. Noda, Y. Toyoda, H. Itakura, Y. Yazaki, S. Horiuchi, K. Takahashi, J. K. Kruijt, T. J. C. van Berkel, U. P. Steinbrecher, S. Ishibashi, N. Maeda, S. Gordon, and T. Kodama.** 1997. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. Nature **386:**292–296.
42. **Takahashi, K., M. Naito, T. Kodama, H. Suzuki, T. Mori, and A. Matsumoto.** 1992. Expression of macrophage scavenger receptors in various human tissues and atherosclerotic lesions. Clin. Biochem. **25:**365–368.
43. **Tsunoda, S., J. Sierralta, Y. Sun, R. Bodner, E. Suzuki, A. Becker, M. Socolich, and C. S. Zuker.** 1997. A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. Nature **388:**243–249.
44. **van der Geer, P., and T. Hunter.** 1993. Mutation of Tyr697, a GRB2-binding site, and Tyr721, a PI 3-kinase binding site, abrogates signal transduction by the murine CSF-1 receptor expressed in Rat-2 fibroblasts. EMBO J. **12:**5161–5172.
45. **Watanabe, S., T. Itoh, and K.-I. Arai.** 1996. JAK2 is essential for activation of c-fos and c-myc promoters and cell proliferation through the human granulocytic-macrophage colony stimulating factor receptor in BA/F3 cells. J. Biol. Chem. **271:**12681–12686.
46. **Wiktor-Jedrzejczak, W., A. Bartocci, A. W. Ferrante, Jr., A. Ahmed-Ansari, K. W. Sell, J. W. Pollard, and E. R. Stanley.** 1990. Total absence of colony stimulating factor 1 in the macrophage-deficient osteopetrotic op/op mouse. Proc. Natl. Acad. Sci. USA **87:**4828–4832.
47. **Wiktor-Jedrzejczak, W., M. Z. Ratajczak, A. Ptasznik, K. W. Sell, A. Ahmed-Ansari, and W. Ostertag.** 1992. CSF-1 deficiency in the op/op mouse has differential effects on macrophage populations and differentiation stages. Exp. Hematol. **20:**1004–1010.
48. **Witmer-Pack, M. D., D. A. Hughes, G. Schuler, L. Lawson, A. McWilliam, K. Inaba, R. M. Steinman, and S. Gordon.** 1993. Identification of macrophages and dendritic cells in the osteopetrotic (op/op) mouse. J. Cell Sci. **104:**1021–1029.
49. **Wu, H., K. Moulton, A. Horvai, S. Parik, and C. K. Glass.** 1994. Combinatorial interactions between AP-1 and ets-domain proteins contribute to the developmental regulation of the macrophage scavenger gene. Mol. Cell. Biol. **14:**2129–2139.
50. **Zhu, Y., M. Pless, R. Inhorn, B. Mathey-Prevot, and A. D. D'Andrea.** 1996. The murine *DUB-1* gene is specifically induced by the βc subunit of the interleukin-3 receptor. Mol. Cell. Biol. **16:**4808–4817.